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I, SMILJA DRAGOSAVLJEVIC, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002950862 for a patent by BIOSIGNAL PTY LTD as filed on 19 August 2002.

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First day of September 2003

S. Dragosavljevic

SMILJA DRAGOSAVLJEVIC
TEAM LEADER EXAMINATION
SUPPORT AND SALES

AUSTRALIA

Patents Act 1990

Biosignal Pty Ltd

PROVISIONAL SPECIFICATION

Invention Title:

Furanone derivatives and methods of making same

The invention is described in the following statement:

Technical Field

The present invention relates to novel synthesis methods, to the products of such novel methods, and to uses of these products. In particular, the present invention provides methods for the reactions of furanones, in particular fimbrolides, with amines. The invention has particular application in the synthesis of halogenated 1,5-dihydro-pyrrol-2-one, 5-halomethylene substituted 1,5-dihydropyrrol-2-ones (lactam analogues of fimbrolides), 5-amino substituted furanones and 5-aminomethylene-2(5H)-furanones and their synthetic analogues. The invention also relates to novel compounds and uses thereof.

Background Art

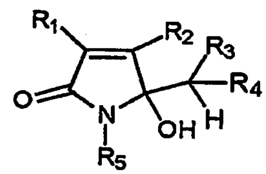
Fimbrolides (halogenated 5-methylene-2(5H)-furanones) possess a wide range of important biological properties including antifungal and antimicrobial properties (see WO 96/29392 and WO 99/53915, the disclosures of which are incorporated herein by cross-reference). These metabolites can be isolated from red marine algae *Delisea fimbriata*, *Delisea elegans* and *Delisea pulchra*.

Despite their biological activity very few hetero atom containing analogues of these molecules have been reported in the literature. The majority of the published syntheses of fimbrolides focus on the preparation of naturally occurring fimbrolides themselves. Recently we have developed methods that yield both the natural and unnatural fimbrolides in good yields (see WO 99/54323 and WO 0200639 the disclosure of which is incorporated herein by cross-reference).

We have now found that, surprisingly, fimbrolides undergo reactions with amines under mild conditions. We have found this discovery to be particularly useful in the synthesis of 5-hydroxy-5-alkyl substituted 1,5-dihydro-pyrrol-2-one, 5-amino-5-alkyl substituted 2(5H)-furanones and 5-aminomethylene substituted 2(5H)-furanones. Furthermore 5-hydroxy-5-halomethyl substituted 1,5-dihydro-pyrrol-2-one generated under these conditions can be dehydrated to yield 5-halomethylene substituted 1,5-dihydropyrrol-2-ones (lactam analogues of fimbrolides), and the 5-amino-5-bromomethyl substituted 2(5H)-furanones can be dehydrobrominated to yield a range of 5-aminomethylene substituted 2(5H)-furanones. These furanones can be further functionalised to yield a range of novel analogues.

Summary of the Invention

In a first aspect, the present invention provides a method for the preparation of compound of formula II



II

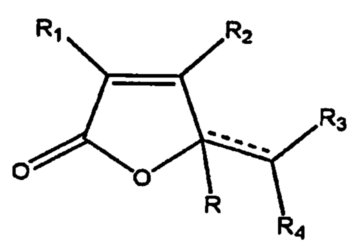
wherein R_1 and R_2 are independently selected from the group H, halogen, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic;

R_3 and R_4 are independently selected from the group H, halogen, alkyl, alkoxy, aryl or arylalkyl;

R_5 is selected from the group consisting of H, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic, or

forms part of an amino acid, is an oligomer, a polymer, a substrate or a surface,

the method comprising reacting a compound of formula I



I

wherein R_1 and R_2 are independently H, halogen, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic;

R_3 and R_4 are independently H, halogen, alkyl, alkoxy, aryl or arylalkyl; and R is hydroxy, halogen; and "- - - -" represents a single bond or a double bond, provided that at least one of R_1, R_2, R_3 and R_4 is halogen,

with a compound of formula R_5NH_2

wherein R_5 is selected from the group consisting of H, alkyl, hydroxy, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic, or

forms part of an amino acid or,

is an oligomer, a polymer, a substrate or a surface.

The reaction may optionally be carried out in the presence of solvent.

Preferably, in the compound of formula II, at least one of R_1, R_2, R_3 and R_4 is halogen.

In the structural formulae described herein, a particular geometry is not to be taken as specified. For example, the formulae covers both Z- and E-isomers.

R_5 may be a residue of a natural or synthetic compound. R_5 may be a biological or non-biological compound. For example, R_5 may be a coenzyme or cofactor. R_5 may be an oligomer or a polymer, which may be biological or synthetic. For example, the oligomer or polymer may be a peptide or polyamide. The polymer may be a protein, for example, an enzyme or a receptor. R_5 may be an oligomer or polymer comprising nucleic acid residues. The polymer may be a polynucleotide, for example, DNA or RNA.

R_5 may be a surface or substrate with which the nitrogen atom of compound II is associated. The association may be chemical bonding, for example covalent bonding. The surface or substrate may be biological or synthetic.

The term "alkyl" is taken to mean both straight chain alkyl groups such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tertiary butyl, and the like. Preferably the alkyl group is a lower alkyl of 1 to 6 carbon atoms. The alkyl group may optionally be substituted by one or more groups selected from alkyl, cycloalkyl, alkenyl, alkynyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxy, alkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino,

acyloxy, alkylsulfonyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphono and phosphinyl.

The term "alkoxy" denotes straight chain or branched alkyloxy, preferably C_{1-10} alkoxy. Examples include methoxy, ethoxy, n-propoxy, isopropoxy and the different butoxy isomers.

The term "alkenyl" denotes groups formed from straight chain, branched or mono- or polycyclic alkenes and polyene. Substituents include mono- or poly-unsaturated alkyl or cycloalkyl groups as previously defined, preferably C_{2-10} alkenyl. Examples of alkenyl include vinyl, allyl, 1-methylvinyl, butenyl, iso-butenyl, 3-methyl-2-butenyl, 1-pentenyl, cyclopentenyl, 1-methyl-cyclopentenyl, 1-hexenyl, 3-hexenyl, cyclohexenyl, 1-heptenyl, 3-heptenyl, 1-octenyl, cyclooctenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 3-decenyl, 1,3-butadienyl, 1,4-pentadienyl, 1,3-cyclopentadienyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, 1,3-cycloheptadienyl, 1,3,5-cycloheptatrienyl, or 1,3,5,7-cyclooctatetraenyl.

The term "halogen" denotes fluorine, chlorine, bromine or iodine, preferably bromine or fluorine.

The term "heteroatoms" denotes O, N or S.

The term "acyl" used either alone or in compound words such as "acyloxy", "acylthio", "acylamino" or diacylamino" denotes an alkanoyl, aroyl, heteroyl, carbamoyl, alkoxycarbonyl, alkanesulfonyl, arysulfonyl, and is preferably a C_{1-10} alkanoyl. Examples of acyl include carbamoyl; straight chain or branched alkanoyl, such as formyl, acetyl, propanoyl, butanoyl, 2-methylpropanoyl, pentanoyl, 2,2-dimethylpropanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl; alkoxycarbonyl, such as methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl, t-pentyloxycarbonyl or heptyloxycarbonyl; cycloalkanecarbonyl such as cyclopropanecarbonyl cyclobutanecarbonyl, cyclopentanecarbonyl or cyclohexanecarbonyl; alkanesulfonyl, such as methanesulfonyl or ethanesulfonyl; alkoxysulfonyl, such as methoxysulfonyl or ethoxysulfonyl; heterocycloalkanecarbonyl; heterocyclyoalkanoyl, such as pyrrolidinylacetyl, pyrrolidinylpropanoyl, pyrrolinylacetyl, pyrrolylacetyl, pyrrolidinylbutanoyl, pyrrolidinylpentanoyl, pyrrolidinylhexanoyl or thiazolidinylacetyl; heterocyclylalkenoyl, such as heterocyclylpropenoyl, heterocyclylbutenoyl, heterocyclylpentenoyl or heterocyclylhexenoyl; or heterocyclylglyoxyloyl, such as, thiazolidinylglyoxyloyl or pyrrolidinylglyoxyloyl.

The term "fluorophilic" is used to indicate the highly attractive interactions between certain groups, such as highly fluorinated alkyl groups of C4-C10 chain length, towards perfluoroalkanes and perfluoroalkane polymers.

The term "amino acid" as used herein includes any compound having at least one amino group and at least one carboxyl group. The amino acid may be a naturally occurring amino acid or it may be a non-naturally occurring amino acid.

The amines used in this invention may be soluble in the reaction medium or insoluble in the reaction medium. Examples of soluble amines include ammonia, alkyl-, aryl-, arylalkyl-, and heterocyclic amines.

Examples of insoluble amines include basic amine resins and amine containing biological and synthetic polymers.

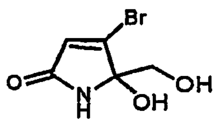
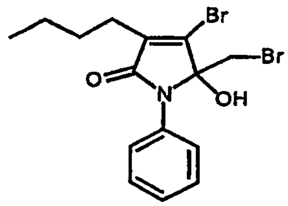
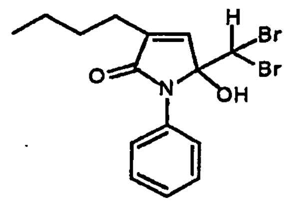
The reaction may be performed in the presence or absence of a solvent. The solvent may be any suitable solvent. Preferable solvents in the present invention include alkyl acetates, aromatic hydrocarbons, chlorinated alkanes, cyclic or open chain ethers such as tetrahydrofuran, diethyl ether, dioxane, and C1-C3 acids. More preferably, the solvents are aromatic hydrocarbons and chlorinated alkanes. Most preferably, the solvent is dichloromethane, as well as dichloroethane and trichloroethane.

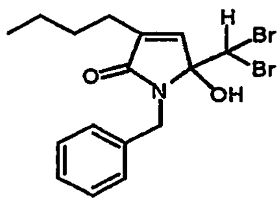
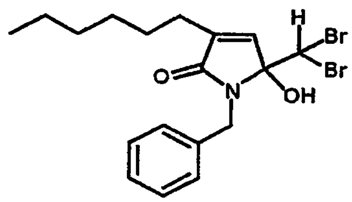
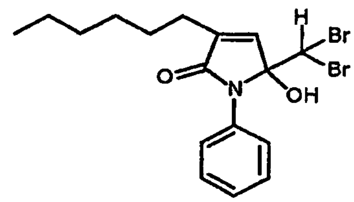
The reaction is preferably carried out at mild temperatures. Preferably the cyclisation reaction is performed at a temperature in the range of 20-150^{0}C.

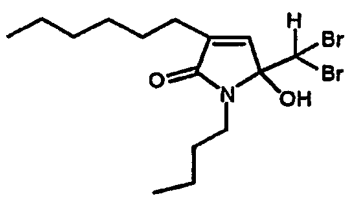
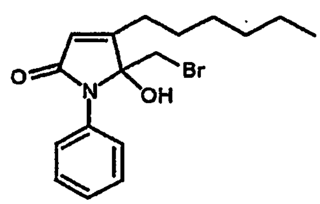
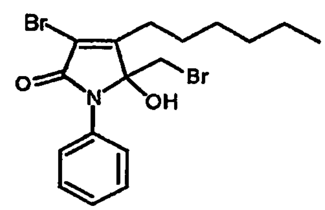
Where a solvent is present, the cyclisation may be performed at reflux temperature, for example, at the reflux temperature of dichloromethane. Optionally the reaction may be carried out below reflux temperature under pressure.

The reaction time may vary from about 2 hours to 12 hours or more and is typically about 2 hours or more. It will be appreciated that reaction conditions may be varied depending upon the individual nature of the substrate and the desired rate of the reaction.

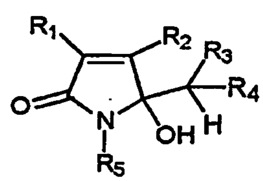
Non-limiting examples of compounds of formula II, which may be described as 5-alkyl-5-hydroxy substituted 1,5-dihydro-pyrrol-2-ones, that can be synthesised by the method of the invention include:

In a second aspect, the present invention provides a compound of formula II:



II

wherein R_1 and R_2 are independently H, halogen, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic;

R_3 and R_4 are independently H, halogen, alkyl, alkoxy, aryl or arylalkyl;

R_5 is selected from the group consisting of H, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally

interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic, or

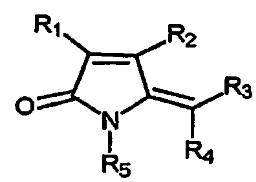
forms part of an amino acid or,

is an oligomer, a polymer, a substrate or a surface.

Particularly preferred are compounds of formula II in which at least one of R_1, R_2, R_3 and R_4 is halogen;

The inventors have found the 5-alkyl-5-hydroxy substituted 1,5-dihydro-pyrrol-2-one of formula II can be dehydrated to yield a range of 5-(halomethylene)- 1,5-dihydro-pyrrol-2-one, 5-(dihalomomethylene)-1,5-dihydro-pyrrol-2-one.

Accordingly in a third aspect, the present invention provides a method for the dehydration of a compound of formula II above, to prepare a compound of formula III;



III

wherein R_1 and R_2 are independently selected from H, halogen, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic;

R_3 and R_4 are independently selected from H, halogen, alkyl, alkoxy, aryl or arylalkyl; and R_5 is a defined above,

the method comprising contacting a compound of formula II with a dehydrating agent.

Preferably at least one of R_1, R_2, R_3 and R_4 in formula III is halogen;

Examples of suitable dehydrating agents include phosphorus pentoxide, silica gel, molecular sieves, alumina, acidic resins and polymers, phosphorus oxychloride, acetic anhydride, N,N'-dicyclohexylcarbodiimide (DCC), trifluoroacetic acid, sulfuric acid, trifluoroacetic anhydride, trifluorosulfonic acid anhydride (triflic anhydride).

Preferably dehydration is carried out using phosphorus pentoxide in the presence of a solvent. The solvent may be any suitable solvent. Preferable

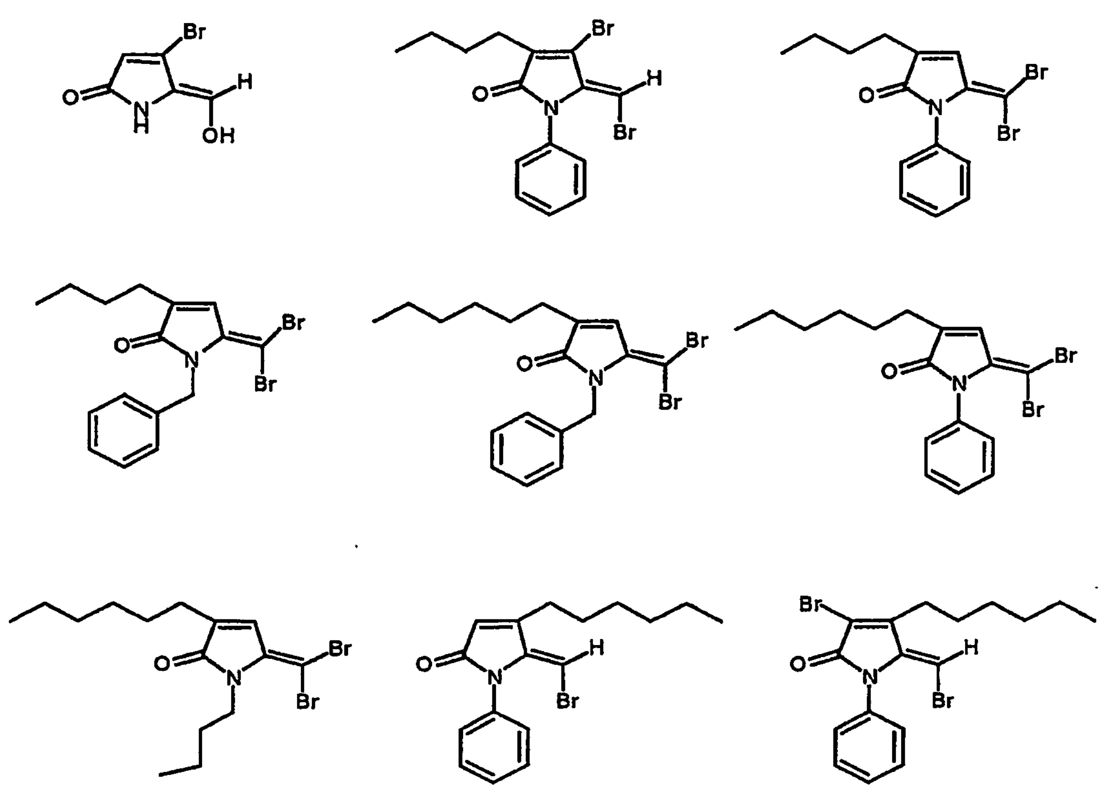
solvents in the present invention include alkyl acetates, aromatic hydrocarbons, chlorinated alkanes, tetrahydrofuran, diethyl ether, dioxane and C1-C3 acids. More preferably, the solvents are aromatic hydrocarbons and chlorinated alkanes. Most preferably, the solvent is dichloromethane, as well as dichloroethane and trichloroethane.

The reaction is preferably carried out at mild temperatures. Preferably the dehydration reaction is performed at a temperature in the range of from about 20-150^{0}C.

Where a solvent is present, the cyclisation may be performed at reflux temperature of the solvent, for example, at the reflux temperature of dichloromethane.

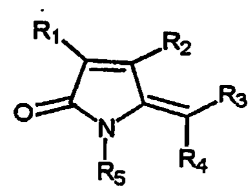
The reaction time may range from about 2 hours to 12 hours or more and is typically about 2 hours or more. It will be appreciated that reaction conditions may be varied depending on the individual nature of the substrate and the desired rate of the reaction.

Non-limiting examples of furanones (III) that can be synthesised by this procedure are listed below.



We believe that the 1,5-dihydro-pyrrol-2-ones prepared of formula III are novel compounds.

Thus, in a fourth aspect, the present invention provides a compound of formula III:



III

wherein R_1 and R_2 are independently selected from H, halogen, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic;

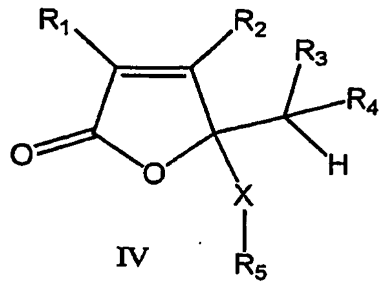
R_3 and R_4 are independently selected from H, halogen, alkyl, alkoxy, aryl or arylalkyl; and

R_5 is as defined above.

Preferably at least one of R_1, R_2, R_3 and R_4 is halogen.

Furthermore the present inventors have also found that furanones of formula (I) when treated with certain amines can yield 5-amino substituted or 5-aminomethylene substituted furanones. Alternatively, the compounds of formula I can be treated with an alcohol to yield 5' alkoxy substituted furanones. For example when 4-bromo-5-bromomethylene-2(5H)-furanone was treated with aniline it gave 4-bromo-5-phenylaminomethylene-2(5H)-furanone in good yields. In contrast, the reaction of 4-bromo-5-bromomethylene-2(5H)-furanone with benzyl amine, gave the corresponding 5-benzylamino-4-bromo-5-bromomethyl-2(5H)-furanone.

Accordingly, in a fifth aspect, the present invention provides a method for the preparation of a compound of formula IV



IV

wherein R_1 and R_2 are independently selected from H, halogen, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic;

R_3 and R_4 are independently selected from H, halogen, alkyl, alkoxy, aryl or arylalkyl; wherein; and

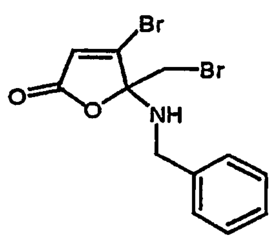
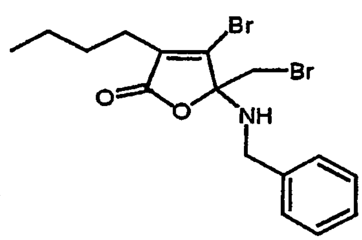
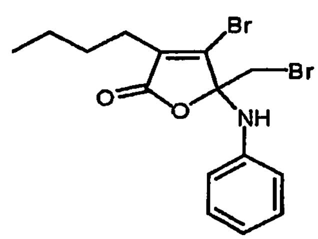
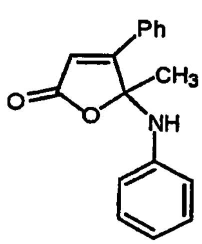
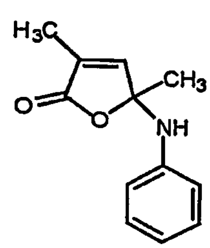
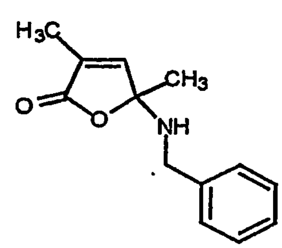
R_5 is as defined above,

X is O or NR_6, where R_6 may be R_1,

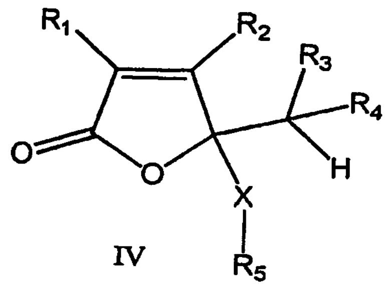
the method comprising reacting a compound of formula I wherein R_3 is a hydrogen and "------" represents a double bond.

Preferably at least one of R_1, R_2, R_3 and R_4 is halogen. Preferably R_6 is H.

Representative examples of furanones (IV) that can be synthesised by this procedure are listed below.

In yet a sixth aspect, the present invention provides a compound of formula IV



IV

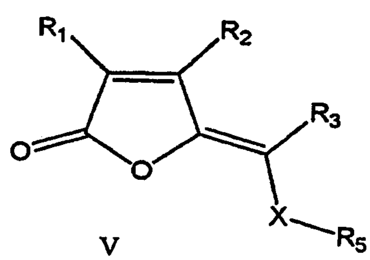
wherein R_1 and R_2 are independently selected from H, halogen, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic;

R_3 and R_4 are independently selected from H, halogen, alkyl, alkoxy, aryl or arylalkyl; and

R_5 and X are as defined above.

Preferably, at least one of R_1, R_2, R_3 and R_4 is halogen.

Accordingly a seventh aspect, the present invention provides for a method for preparation of a compound of formula V.



V

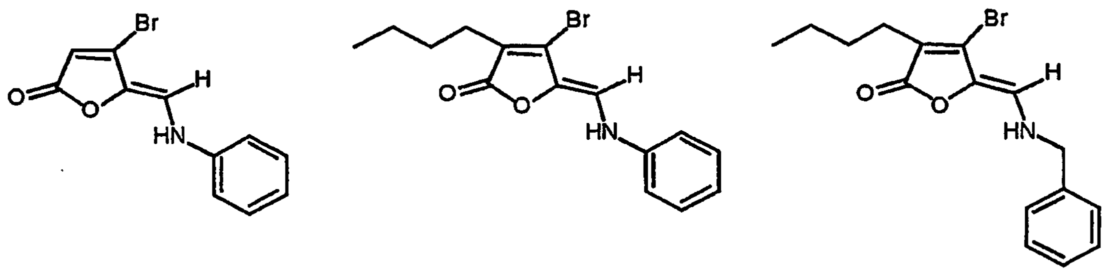
wherein R_1 and R_2 are independently selected from H, halogen, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic;

R_3 is selected from H, halogen, alkyl, alkoxy, aryl or arylalkyl;

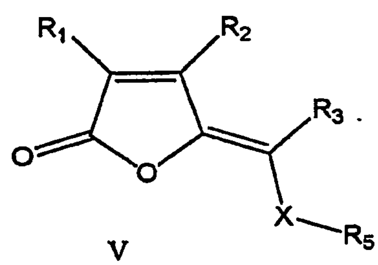
X is O or NR_6, where R_6 is as defined above; and

R_5 is as defined above.

Non-limiting examples of furanones of formula (V) that can be synthesised by this procedure are listed below.



In an eighth aspect, the present invention provides a compound of formula V:



V

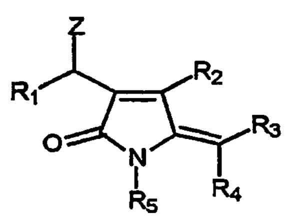
wherein R_1 and R_2 are independently selected from H, halogen, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic;

R_3 is selected from H, halogen, alkyl, alkoxy, aryl or arylalkyl;

X is O or NR_6, where R_6 is as defined above; and

R_5 is as defined above.

In yet a ninth aspect the present invention provides a compound of formula (VI):



VI

wherein R_1 and R_2 are independently selected from H, halogen, alkyl, alkoxy, oxoalkyl, alkenyl, aryl or arylalkyl whether unsubstituted or substituted, optionally interrupted by one or more hetero atoms, straight chain or branched chain, hydrophilic or fluorophilic;

R_3 and R_4 are independently selected from H, halogen, alkyl, aryl or arylalkyl;

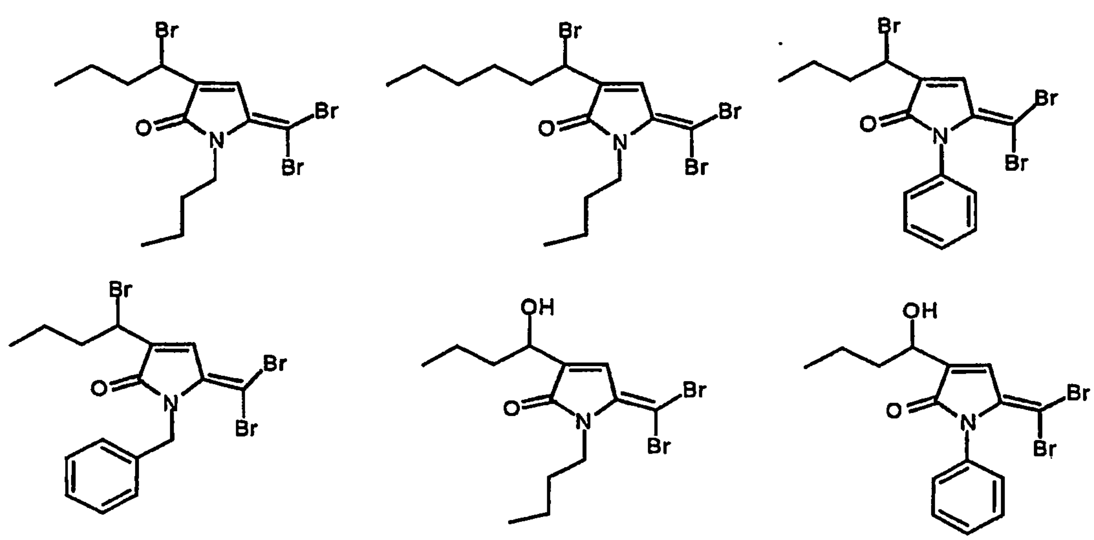
R_5 is defined as above ; and

Z is selected from the group R_2, halogen, $OC(O)R_2$, =O, amine azide, thiol, R_2, mercaptoaryl, arylalkoxy, mercaptoarylalkyl, $SC(O)R_2$, $OS(O)_2R_2$, $NHC(O)R_2$, $=NR_2$ or NHR_2.

The compounds of formula VI may be prepared by functionalizing a fimbrolide of formula (III) wherein, R_1, R_2, R_3 and R_4 are as defined above, with a reagent described in WO 99/54323, (the disclosure of which is incorporated herein by cross-reference).

Reagents for introduction and manipulation of the Z group include halogenating and oxidising agents (N-halosuccinimide, lead tetraacetate, selenium dioxide, Jones reagent), nucleophiles (including organic metal carboxylates, organic alcohols, dimethyl sulfoxide and organonitriles) and electrophiles including (organic acids, isocyanates, carboxylic or sulfonic acid halides and diethylaminosulfur trifluoride).

Non-limiting examples of furanones of formula (VI) that can be synthesised by this procedure are listed below.



In a tenth aspect, the present invention provides an oligomer or polymer formed by oligomerising or polymerising a compound of formula II – VI, described herein directly or with one or more other monomers.

The one or more other monomer may be any suitable polymerisable copolymer e.g. acrylate ester such as alkyl, hydroxyalkyl, aminoalkyl, or substituted aryl acrylates or methacrylates, crotonates, substituted or unsubstituted acrylonitriles, vinyl alcohols or acetates, styrene and siloxanes.

In an eleventh aspect, the present invention provides incorporation of compounds produced by the methods according to the first, third, fifth, seventh, ninth, or tenth aspects either in surface coatings or polymers through the newly introduced functionality on the alkyl chain or the alkyl chain itself via direct polymerisation or copolymerisation with suitable monomers.

In an twelfth aspect, the present invention provides a compound produced by the method according to the first, third, fifth, seventh, ninth, or eleventh aspects of the present invention.

In a thirteenth aspect, the present invention provides the use of a compound produced according to the present invention. The present inventors have found that many of the 1,5-dihydro-pyrrol-2-one derivatives and furanones having the formula (II), (III), (IV), (V) and (VI) have antimicrobial and/or antifouling properties. Accordingly, the fimbrolide derivatives are suitable for use as antimicrobial and/or antifouling agents.

Thus in a fourteenth aspect, the present invention provides methods of use of compounds of formula (II), (III), (IV), (V) and (VI) in medical, scientific and/or biological applications.

For these and other applications, the compounds of the present invention may be formulated as a composition.

In a fifteenth aspect, the present invention provides a composition comprising at least one compound of formula (II), (III), (IV), (V) or (VI).

The compositions of the third aspect of the invention may be in any suitable form. The composition may include a carrier or diluent. The carrier may be liquid or solid. For example, the compositions may be in the form of a solution or suspension of at least one of the compounds in a liquid. The liquid may be an aqueous solvent or a non-aqueous solvent. The liquid may consist of or comprise a one or more organic solvents. The liquid may be an ionic liquid. Particular examples of carrier or diluents include, but are not limited to,

water, polyethylene glycol, propylene glycol, cyclodextrin and derivatives thereof.

The composition may be formulated for delivery in an aerosol or powder form.

The composition may include organic or inorganic polymeric substances. For example, the compound of the invention may be admixed with a polymer or bound to, or adsorbed on to, a polymer.

When the composition is to be formulated as a disinfectant or cleaning formulation, the composition may include conventional additives used in such formulations. Non-limiting examples of the physical form of the formulations include powders, solutions, suspensions, dispersions, emulsions and gels.

Formulations for pharmaceutical uses may incorporate pharmaceutically acceptable carriers, diluents and excipients known to those skilled in the art. The compositions make be formulated for parenteral or non-parenteral administration. The composition of the invention may be formulated for methods of introduction including, but not limited to, topical, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, ophthalmic, and oral routes. It may be formulated for administration by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration may be localized or systemic. The composition may be formulated for intraventricular and intrathecal injection. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In certain preferred embodiments the composition further comprises other active agents such as antibiotics and cleaning agents.

In a sixteenth aspect, the present invention provides a method of treating an infection in a human or animal subject the method comprising administration to the subject of an effective amount of the compound of the invention.

The treatment may therapeutic and/or prophylactic.

The compounds of the present invention can act as quorum sensing inhibitors and therefore find use in any application where such as effect is desired. For example, the compounds of the present invention may have use in preventing the establishment and expression of virulence by microorganisms through the inhibition of quorum sensing systems and/or other extracellular

systems (eg see, International patent application No. PCT/AU01/01621, the disclosure of which is incorporated herein in its entirety).

The method of the sixteenth aspect may be used to treat an infection or condition in a subject that is characterised by biofilm formation.

The condition may be cystic fibrosis. The infection may be that resulting from a skin infection, burn infection and/or wound infection. The method and composition of the invention may be particularly suitable for the treatment of infection in immuno compromised individuals.

In yet a seventeenth aspect, the present invention provides a method for treating biofilm formation on a surface by contacting the surface with a compound in accordance with the present invention.

The term "surface" as used herein relates to any surface which may be covered by a biofilm layer. The surface may be a biological (eg tissue, membrane, skin etc) or non-biological surface.

The surface may be that of a natural surface, for example, plant seed, wood, fibre etc.

The surface may be any hard surface such as metal, organic and inorganic polymer surface, natural and synthetic elastomers, board, glass, wood, paper, concrete, rock, marble, gypsum and ceramic materials which optionally are coated, eg with paint, enamel etc; or any soft surface such as fibres of any kind (yarns, textiles, vegetable fibres, rock wool, hair etc.); or porous surfaces; skin (human or animal); keratinous materials (nails etc.). The hard surface can be present in process equipment or components of cooling equipment, for example, a cooling tower, a water treatment plant, a dairy, a food processing plant, a chemical or pharmaceutical process plant. The porous surface can be present in a filter, eg. a membrane filter.

Particular examples of surfaces that may be treated in accordance with the invention include, but are not limited to, toilet bowls, bathtubs, drains, highchairs, counter tops, vegetables, meat processing rooms, butcher shops, food preparation areas, air ducts, air-conditioners, carpets, paper or woven product treatment, nappies(diapers), personal hygiene products (eg sanitary napkins) and washing machines. The cleaning composition may be in the form of a toilet drop-in or spray-on devices for prevention and removal of soil and under rim cleaner for toilets. The compositions and methods of the present invention also have applications in cleaning of Industrial surfaces such as floors, benches, walls and the like and these and other surfaces in medical

establishments such as hospitals (eg surfaces in operating theatres), veterinary hospitals, and in mortuaries and funeral parlours.

A compound of the invention may be incorporated into epidermal bandages and lotions. Alternatively, the compounds of the invention may be incorporated into cosmetic formulations, for example, aftershave lotions.

Compositions of the present invention may be in the form of an aqueous solution or suspension containing a cleaning-effective amount of the active compound described above. The cleaning composition may be in the form of a spray, a dispensable liquid, or a toilet tank drop-in, under-rim product for prevention, removal and cleaning of toilets and other wet or intermittently wet surfaces in domestic or industrial environments.

The compositions of the present invention may additionally comprise a surfactant selected from the group consisting of anionic, non-ionic, amphoteric, biological surfactants and mixtures thereof. Most preferably, the surfactant is sodium dodecyl sulfate.

One or more adjuvant compounds may be added to the cleaning solution of the present invention. They may be selected from one or more of biocides, fungicides, antibiotics, and mixtures thereof to affect planktonics. pH regulators, perfumes, dyes or colorants may also be added.

By "cleaning-effective" amount of active compound, it is meant an amount of the compound which is necessary to remove at least 10% of bacteria from a biofilm as determined by a reduction in numbers of bacteria within the biofilm when compared with a biofilm not exposed to the active compound.

The cleaning methods of the present invention are suitable for cleaning surfaces. They may be used to treat hard, rigid surfaces such as drain pipes, glazed ceramic, porcelain, glass, metal, wood, chrome, plastic, vinyl and formica or soft flexible surfaces such as shower curtains, upholstery, laundry and carpeting. It is also envisioned that both woven and non woven and porous and non-porous surfaces would be suitable.

In other embodiments of the present invention, the composition of the invention may be formulated as a dentifrice, a mouthwash or a composition for the treatment of dental caries. The composition may be formulated for acne treatment or cleaning and disinfecting contact lenses (eg as a saline solution).

The method of the invention may be used to treat implanted devices that are permanent such as an artificial heart valve or hip joint, and

those that are not permanent such as indwelling catheters, pacemakers, surgical pins etc. The method may further be used in situations involving bacterial infection of a host, either human or animal, for example in a topical dressing for burn patients. An example of such a situation would be the infection by *P. aeruginosa* of superficial wounds such as are found in burn patients or in the lung of a cystic fibrosis patient.

In other forms, the present invention can be used to treat integrated circuits, circuit boards or other electronic or microelectronic devices.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Modes for Carrying Out the Invention

The invention is further described in and illustrated by the following examples. The examples are not to be construed as limiting the invention in any way.

EXPERIMENTAL DETAILS

General. Melting points are uncorrected. Microanalyses were performed by Dr H.P. Pham of The University of New South Wales Microanalytical Laboratory. ^{1}H NMR spectra were obtained in $CDCl_3$ on a Bruker AC300F (300 MHz) or a Bruker DMX500 (500 MHz) spectrometer. ^{13}C NMR were obtained in the same solvent on a Bruker AC300F (75.5 MHz) or a Bruker DMX500 (125.8 MHz) spectrometer. Chemical shifts were measured on the δ scale internally referenced to the solvent peaks: $CDCl_3$ (δ 7.26, δ 77.04). Ultraviolet spectra were measured on an Hitachi U-3200 spectrophotometer and refer to solutions in absolute MeOH. Infrared spectra were recorded on a Perkin-Elmer 298 or a Perkin-Elmer 580B spectrophotometer and refer to paraffin mulls. The electron impact mass spectra were recorded on an VG Quattro mass spectrometer at 70eV ionisation voltage and 200°C ion source temperature. FAB spectra were recorded on an AutoSpecQ mass spectrometer. Column chromatography was carried out using Merck silica gel 60H (Art. 7736), whilst preparative thin layer chromatography was performed on 2 mm plates using Merck silica gel 60GF$_{254}$ (Art. 7730).

3-Butyl-5-dibromomethyl-5-hydroxy-1-phenyl-1,5-dihydropyrrol-2-one

A solution of 3-butyl-5-dibromomethylene-2(5H)furanone (0.20 g; 0.65 mmol) in aniline (5 ml) was allowed to stand at room temperature for 24 h. The mixture was diluted with dichloromethane (25 ml) and washed with aqueous hydrochloric acid (2M, 20 ml). The organic phase was dried over sodium sulfate and evaporated to yield a yellow viscous oil (0.30 g). The crude product was chromatographed on silica using dichloromethane/ethylacetate (19:1; v:v) as the eluent. The major product, a pale yellow band, was collected and recrystallised from light petroleum to yield 3-butyl-5-dibromomethyl-5-hydroxy-1-phenyl-1,5-dihydropyrrol-2-one as colourless prisms (0.24 g, 92%), m.p. 96-98°C ν_{max} 3211, 2957, 1679, 1597, 1500, 1417, 1117, 1058, 760, 698 cm^{-1}. λ_{max}: 263nm (ε_{max} 2,955), 202 (2,464). ^{1}H n.m.r. δ ($CDCl_3$): 7.54-7.37, m, Ph; 6.82, 1H, s, C4-H; 5.56, 1H, s, -CH_2Br; 3.42, s, C5-OH; 2.43-2.41, m, 2H, C4-chain; 1.64-0.97, m, C4 chain. ^{13}C n.m.r. δ ($CDCl_3$): 13.7, 14.0, 22.3, 46.6, 92.2, 126.7, 127.4, 129.0, 134.0, 135.5, 136.0, 144.3, 169.0.

5-Dibromomethyl-3-hexyl-5-hydroxy-1-phenyl-1,5-dihydropyrrol-2-one

A mixture of 3-hexyl-5-dibromomethylene-2(5H)furanone (0.40g, 1.18 mmol) and aniline (1 ml) in ethanol (6 ml) was refluxed for 3h. The solvent was evaporated off and the residue extracted with dichloromethane (25 ml). The organic phase was washed with aqueous hydrochloric acid (2M, 2 x 20 ml), dried over sodium sulfate and evaporated to yield a semi-solid (0.39 g). The crude product was chromatographed on silica using dichloromethane/ethylacetate (19:1; v:v) as the eluent. The major product, a pale yellow band, was collected and recrystallised from light petroleum to yield 5-dibromomethyl-3-hexyl-5-hydroxy-1-phenyl-1,5-dihydropyrrol-2-one as a semi-crystalline solid (23%), m.p. 43-45° (Found (HRESMS) 451.982217. $C_{17}H_{21}Br_2NO_2Na^+$ (^{79}Br) requires 451.983106). ν_{max}: 3186, 2926, 1680, 1659, 1492, 1372, 1095, 1059, 897, 850, 766, 747, 699, 671 cm^{-1}. λ_{max}: 261nm (ε_{max} 4051), 206 (26.550). ^1H n.m.r. δ ($CDCl_3$): 7.5-7.25, m, 5H, Ph; 6.8, s, 1H, C3-H; 5.5, s, 2H, -$\underline{CH_2}$Ph; 3.77, brs, 1H, C5-OH; 2.44-2.34, m, 2H, C4-chain; 2.03-0.91, 13H, C4-chain. ^{13}C n.m.r. δ ($CDCl_3$): 14.2, 25.3, 29.0, 31.0, 46.6, 92.0, 104.8, 126.7, 127.0, 136.0, 144.0, 169.0, 172.0.

1-Benzyl-3-butyl-5-dibromomethyl-5-hydroxy-1,5-dihydropyrrol-2-one

A solution of 3-butyl-5-dibromomethylene-2(5H)furanone (1.03 g: 3.32 mmol) in benzyl amine (2ml) was allowed to stand at room temperature for 1 h during which time the reaction mixture solidified. The solid was dissolved in dichloromethane (25 ml) and washed with aqueous hydrochloric acid (2M, 20 ml). The organic phase was dried over sodium sulfate and evaporated to yield a yellow viscous oil. The crude product was triturated with light petroleum to yield a white solid (1.0 g; 74%) which was recrystallised from light petroleum to yield 1-benzyl-3-butyl-5-dibromomethyl-5-hydroxy-1,5-dihydropyrrol-2-one as colourless needles, m.p. 92-93°C (Found (HRESMS) m/z 479.974243. $C_{18}H_{21}Br_2NO_3Na^+$ (^{79}Br) requires 479.978123). ν_{max}: 2987, 2953, 2920, 1677, 1650, 1449, 1424, 1069 cm^{-1}. λ_{max}: 207 (88,250). ^1Hn.m.r. δ ($CDCl_3$): 7.4-7.29, m, 5H, Ph; 6.72, s, 1H, C3-H; 5.56, s, 1H, -$CHBr_2$; 3.0, 1H, C-5 OH; 1.54-0.97, m, C-4 chain. ^{13}C n.m.r.δ ($CDCl_3$): 13.7, 22.3, 29.3, 42.6, 46.8, 91.5, 127.6, 128.3, 128.7, 136.9, 137.0, 160.8, 170.6.

1-Benzyl-5-dibromomethyl-3-hexyl-5-hydroxy-1,5-dihydropyrrol-2-one

Method A

A solution of 3-hexyl-5-dibromomethylene-2(5H)furanone (1.03 g: 3.32 mmol) in benzyl amine (2 ml) was stirred at room temperature for 0.5h. Dichloromethane (15 ml) was added to the reaction mixture and the precipitated solid was filtered off. The filtrate was washed with aqueous hydrochloric acid (2M, 20 ml), dried over sodium sulfate and evaporated to yield a yellow viscous oil (0.36g). The crude product was chromatographed on silica using dichloromethane/ethyl acetate (1:19) as the eluent and recrystallised from light petroleum to yield 1-benzyl-5-dibromomethyl-3-hexyl-5-hydroxy-1,5-dihydropyrrol-2-one (0.11g) as colourless needles m.p.105-108°C. (Found (HRESMS) m/z 465.994011. $C_{18}H_{23}Br_2NO_2Na^+$ (^{79}Br) requires 465.998758. ν_{max}: 3195, 2987, 2924, 2858, 1676, 1649, 1425, 1153, 1068, 968, 845, 730, 599 cm^{-1}. λ_{max}: 205 (ε_{max} 7740) nm. ^1H n.m.r.$\delta(CDCl_3)$: 7.39-7.26, m, 5H, Ph; 6.7, s, 1H, C3-H; 5.6, s, 2H, $-CH_2Ph$; 4.54, 2 d, J 15 Hz, two 1H, C5-C<u>H</u>Br_2; 2.89-2.35, m, 2H, C3-chain; 1.60-0.87, m, 13H, C3-chain. ^{13}C n.m.r. $\delta CDCl_3$): 14, 22.45, 25, 27, 28.8, 31.4, 42,5, 46.7, 91.5, 127.6, 128.5, 128.6, 136.6, 136.7, 144.0, 170.0.

Method B

A mixture of 3-hexyl-5-dibromomethylene-2(5H)furanone (1.03 g: 3.32 mmol) and benzyl amine (2ml) in ethanol (5 ml) was stirred at room temperature for 2.5h. The crude product was isolated and purified as described above to yield 1-benzyl-5-dibromomethyl-3-hexyl-5-hydroxy-1,5-dihydropyrrol-2-one in (72%) yield.

1-Butyl-5-dibromomethyl-3-hexyl-5-hydroxy-1,5-dihydropyrrol-2-one

n-Butylamine (0.272 g; 3.72 mmol) was added dropwise to a solution of 5-dibromomethylene-3-hexyl-2(5H)furanone (0.314 g; 0.93 mmol) in CH_2Cl_2 (10 ml). The mixture was stirred at room temperature for 5 hrs. Column chromatography on silica with CH_2Cl_2 followed by CH_2Cl_2/EtOAc (19:1) afforded the major product as a colourless oil (0.20 g) which upon recrystallisation from petrol gave 1-butyl-5-dibromomethyl-3-hexyl-5-hydroxy-1,5-dihydropyrrol-2-one (52%) as colourless needles, m.p. 85-86°. (Found(HRESMS) m/z 432.013664. $C_{15}H_{25}Br_2NO_2Na^+$ (^{79}Br) requires 432.014407). ν_{max}: 3230, 2957, 2859, 1672, 1650, 1458, 1422, 1375, 1270, 1233, 1139, 1079, 1023, 728, 666, 612 cm-1. λ_{max}: 259 (ε_{max} 945), 206 (9658) nm. ^1H n.m.r. δ ($CDCl_3$): 6.68, s, 1H, C4-H; 5.8, s, 1H, C5-OH; 3.5-3.4 and

3.23-3.09, 2m, $-CHBr_2$; 2.33-2.31, m, $-CH_2$-chain; 1.65-0.88, m, 20H, chain. ^{13}C n.m.r. $\delta(CDCl_3)$: 13.6, 20, 22, 25, 27, 29, 30.75, 31, 39, 46.6, 91.4, 136, 144.5, 170.

5-Dibromomethyl-3-hexyl-5-hydroxy-1,5-dihydropyrrol-2-one

Liquid ammonia (5ml) was added to 5-dibromomethylene-3-hexyl-2(5H)furanone (0.50 g; 1.48 mmol) in a sealed tube held in a acetone/liquid nitrogen bath. The reaction mixture was allowed to warm up gradually and kept at room temperature overnight. After gradual evaporation of ammonia the product was extracted with EtOAc (20 ml), washed with water, dried over Na_2SO_4, and evaporated to yield a solid (0.30g). The crude product was purified on a silica column using first CH_2Cl_2 as the eluent followed by EtOAc/MeOH (4:1). The yellow band on solvent removal and crystallisation from petrol afforded a yellow crystalline solid (0.07g) of 5-dibromomethyl-3-hexyl-5-hydroxy-1,5-dihydropyrrol-2-one, m.p. 106-109°C. ^1H n.m.r. $\delta(CDCl_3)$: 6.61; s, 1H, C4-H; 6.26, s, 1H, -NH; 3.2, s, C5-OH; 2.28-2.23, m, $-CH_2$, chain; 1.55-0.91, m, 11 H, chain. ^{13}C n.m.r. $\delta(CDCl_3)$: 13.9, 22, 25.5, 27, 29, 31.4, 129, 140, 142, 170.5.

4-bromo-5-hydroxy-5-hydroxymethyl-1,5-dihydropyrrol-2-one

A suspension of 4-bromo-5-bromomethylene-2(5H)-furanone (1.30g, 5.16 mmol) in aqueous ammonia solution (20% w/w) was stirred at room temperature for 1/2 h. During this time a complete dissolution of furanone was observed. The solution was evaporated to dryness in vacuo at ca 35-40 °C, and finally under high vacuum at room temperature. The resulting solid (1.70 g) was recrystallised from ethanol to yield 4-bromo-5-hydroxy-5 -hydroxymethyl-1,5-dihydropyrrol-2-one as colourless granules (1.0 g). m.p. 140-142°C (decomp ν_{max}: 3259, 3100, 2949, 1667, 1592, 1419, 1370, 1152, 1076, 981, 872, 563 cm^{-1}. λ_{max} 220 (ε_{max} 6077). ^1H n.m.r.. δ $(CDCl_3)$: 8.09, s, -NH; 6.22, d, 2 Hz; 4.97, t, 2 Hz, $-CH_2OH$; 3.37, q, J 2 Hz; 2.48, d, 2Hz, $-\underline{CH_2}OH$. ^{13}C n.m.r. δ $(CDCl_3)$: 69.6, 84.3, 132,8, 152,3, 174.1.

4-Bromo-3-hexyl-5-hydroxy-5-hydroxymethyl-1,5-dihydropyrrol-2-one

A suspension of 4-bromo-3-hexyl-5-bromomethylene-2(5H)-furanone (0.50 g; 1.48 mmol) in aqueous ammonia solution (30 mls; 28%) was stirred at room temperature for 2h, during which time the solid completely dissolved. The

solution was evaporated to dryness, and the residue extracted with dichloromethane (25 ml). The organic phase was dried over anhydrous sodium sulfate and evaporated to yield a red viscous oil. Chromatography on silica using ethyl acetate followed by ethyl acetate/methanol (4:1). gave a solid which upon recrystallisation from light petroleum yielded 4-bromo-3-hexyl-5-hydroxy-5-hydroxymethyl-1,5-dihydropyrrol-2-one as colourless granules (0.16g; 36%), m.p. 134-135 °. ν_{max}: 3304, 3256, 3185, 2961, 1670, 1589, 1441, 1350, 1136, 1069, 983 cm-1; λ_{max}: 221 (ε_{max} 6,678), 196 (3,415) nm.

5-Ethyl-5-hydroxy-4-methyl-1,5-dihydropyrrol-2-one

A mixture of 5-ethylidene-4-methyl-2(5H)furanone (0.02 g; 0.162 mmol) in aqueous ammonia solution (5 ml; 28% w/w) was stirred at room temperature for 1.5 h during which time all of the furanone dissolved. The solution was evaporated in vacuo to dryness leaving 5-ethyl-5-hydroxy-4-methyl-1,5-dihydropyrrol-2-one as a white solid (0.015g; 65%), m.p. 182-186°C. (Found(HRESMS) m/z 164.067448. $C_6H_{11}NO_2Na^+$ requires 160.06815). ν_{max}: 3204, 2980, 1698, 1664, 1633.5, 1445, 1157, 1080, 1016, 983, 852, 769, 578. λ_{max} 207 (ε_{max}23,180) nm. ^{1}H n.m.r. δ(DMSO)-d_6) 7.97, s, 1H, -NH; 5.55, s, 1H, C4-H,; 3.18, s, 1H, C5-OH; 1.79, s, 3H, C3-Me; 1.69-1.52, m, 2H, C5-CH2-Me. ^{13}C n.m.r.δ(DMSO-d_6): 7.9, 11.9, 29.2, 90.2, 121,7, 162, 171.6.

5-Ethyl-5-hydroxy-4-methyl-1-phenyl-1,5-dihydropyrrol-2-one

A solution of 5-ethylidene-4-methyl-2(5H)furanone (0.31 g; 2.5 mmol) in aniline (0.26 g; 2.75 mmol) was left to stand at r.t. for 3 hrs, during which time a solid precipitated from the reaction. The reaction mixture was triturated with CH_2Cl_2/petrol (1:1) and the solid filtered and recrystallised from EtOAc/petrol to yield and E/Z mixture of 5-ethyl-5-hydroxy-4-methyl-1-phenyl-1,5-dihydropyrrol-2-one as colourless crystals (70%); m.p. 97-100°. ^{1}H n.m.r. δ(DMSO-d_6) 10.1, s, 1H, -NHPh; 7.58, t, 3H, C3-Me and 7.28, t, C3-Me; 6.08, s, 1H, C3-H; 3.28, 2 s, 2 C5-Me. ^{13}C n.m.r. δ($CDCl_3$): 20.9, 72.7, 95.65, 119.6, 124, 129, 139, 143, 163, 170, 172.5.

1-Benzyl-5-ethyl-5-hydroxy-4-methyl-1,5-dihydropyrrol-2-one

A solution of 5-ethylidene-4-methyl-2(5H)furanone (0.124 g; 10 mmol) in benzylamine (0.128g; 12 mmol) was the left to stand at room temperature for 72 hrs, during which time a solid precipitated from the reaction. The reaction

mixture was triturated with CH_2Cl_2/petrol (1:3) and the precipitated solid was filtered and recrystallised from EtOAc/petrol to yield an E/Z mixture of 1-benzyl-5-ethyl-5-hydroxy-4-methyl-1,5-dihydropyrrol-2-one as colourless crystals m.p. 129-132° (70%). ν_{max}: 3247, 3082, 2964, 1669, 1638, 1496, 1353, 1101, 1053, 902, 708 cm^{-1}. λ_{max}: 276 (ε_{max} 2,101), 237 (16,321), 243 (39,646) nm.. ^1H n.m.r. $\delta(CDCl_3)$: 7.4-7.24, m, 5H, Ph; 5.79, s, 1H, C3-H; 4.46, 2 d, J 15 Hz, $-CH_2Ph$; 3.81, brs, C5-OH; 1.92, s, C4-Me; 1.83-168, m, C5-$C\underline{H_2}Me$; 0.34, t, J 7.51 Hz, C5-$CH_2\underline{Me}$. ^{13}C n.m.r. $\delta(CDCl_3)$: 6.8, 11.85, 26, 41.9, 94, 122, 127, 128, 128.5, 138, 159, 170.

5-Aminomethyl-4-heptyl-5-hydroxy-1,5-dihydropyrrol-2-one

5-Bromo-5-bromomethyl-4-heptyl-2(5H)furanone (0.50 g; 1.47 mmol) was dissolved in liquid ammonia in a sealed tube, and left to stand at room temperature for 72 h. Ammonia was allowed to gradually evaporated leaving behind a yellow crystalline solid. The solid was dissolved in hot ethylacetate (ca 25) ml to remove ammonium bromide and the clear filtrate was concentrated to a small volume (ca 7 ml), to yield 5-aminomethyl-4-heptyl-5-hydroxy-1,5-dihydropyrrol-2-one as a crystalline solid, (0.1 g; 34%); m.p. 176°C. ν_{max}: 3370, 3248, 2956, 2926, 2855, 1674, 1627, 1469, 1350, 1227,1095, 1082, 954, 855 cm^{-1}. λ_{max}: 208 (ε_{max} 6845), 291 (2754) nm. ^1H n.m.r. $\delta(CDCl_3)$: 7.53, s, -NH; 5.49, d, C5-$CH_2N\underline{H_2}$; 3.35, 3H,m, -C5-$\underline{OH}$ and $-CH_2\underline{NH_2}$; 2.23-2.0, m, chain; 1.52-0.85, m, 13H, chain. ^{13}C $\delta(CDCl_3)$: 14, 22.4, 26, 26.5, 28.9, 29, 31.6, 66, 73, 78, 120.5, 167.5, 171.6.

5-Bromomethyl-4-heptyl-5-hydroxy-1-phenyl-1,5-dihydropyrrol-2-one

5-Bromo-5-bromomethyl-4-heptyl-2(5H)furanone (0.51g; 1.5 mmol) was dissolved in dry aniline (5 ml). The mixture soon solidified; it was allowed to stand at room temperature for 24 h. Dichloromethane (25 mls) was added to the mixture and the organic phase was washed with aqueous hydrochloric acid (2M) and brine. The dried (Na_2SO_4) organic phase was evaporated to yield a yellow solid (0.50 g; 91%). Recrystallisation from light petroleum gave 5-bromomethyl-4-heptyl-5-hydroxy-1-phenyl-1,5-dihydropyrrol-2-one as colourless needles, m.p. 152-154°C. ν_{max}: 3194, 2956, 1930, 2854, 1676, 1626, 1589, 1502, 1494, 1393, 1246, 1141, 836, 758, 692 cm^{-1}. λ_{max}: 257(ε_{max} 3947), 202 (27,313) nm. ^1H n.m.r. $\delta(CDCl_3)$: 7.55-7.26, m, 5H, Ph; 5.79, s, C3-H; 4.52, 1H, C5-OH; 3.39, d, 2H, C5-CH_2Br; 2.27-2.12, m, 2H, chain; 1.6-0.91,

m, 13H, chain. ^{13}C n.m.r. δ($CDCl_3$): 14, 22.5, 25.6, 25.8, 29, 29.2, 30.4, 31.6, 121.6, 126, 126.7, 130, 134.6, 163, 170.5.

1-Benzyl-5-bromomethyl-4-heptyl-5-hydroxy-1,5-dihydropyrrol-2-one

A mixture of 5-bromo-5-bromomethyl-4-heptyl-2(5H)furanone (0.51g, 1.5 mmol) in benzylamine (0.30g; 2.82 mmol) in ethanol (6 ml) was stirred at room temperature for 1h. Dichloromethane (25 ml) was added to the reaction mixture and the organic phase was washed with aqueous hydrochloric acid (2M) followed by brine. After drying over sodium sulfate, the solvent was evaporated in vacuo to yield 1-benzyl-5-bromomethyl-4-heptyl-5-hydroxy-1,5-dihydropyrrol-2-one as a viscous oil (0.52 g; 97%) which solidified on standing in the fridge. Colourless needles from light petroleum; m.p. 94-96°.ν_{max}: 3270, 3062, 3033, 2957, 2854, 1667, 1637, 1607, 1496, 1416, 1335, 1297, 1257, 1190, 1161, 1140, 1109, 1030, 950, 884, 865, 769 cm^{-1}. λ_{max}: 251 (ε_{max} 2391), 206 (18,974) nm. ^1H n.m.r. δ($CDCl_3$): 7.36-7.28, m, 5H, Ph; 5.85, s, C4-H; 4.54 and 3,42, 2d, 2H, C5-CH_2Br; 3.42, m, 1H, C5-OH, 2.31-2.15, m, 2H, chain; 1.62-0.88, m, 13 H, chain.^{13}C, n.m.r. δ($CDCl_3$): 14, 22.5, 25.5, 26, 29, 29.2, 30.87, 41.9, 122, 127, 128.3, 137.5, 163, 171.

Synthesis of 3-alkyl-5-halomethylene-1,5-dihydropyrrol-2-one

3-Butyl-5-dibromomethylene-1,5-dihydropyrrol-2-one

Phosphorus pentoxide was added to a solution of 3-butyl-5-dibromomethyl-5-hydroxy-1-phenyl-1,5-dihydropyrrol-2-one in chloroform. The resulting mixture was stirred overnight at room temperature and passed through a pad of Celite. The crude product was chromatographed on silica and recrystallised from light petroleum to yield 3-butyl-5-dibromomethylene-1,5-dihydropyrrol-2-one as orange needles (16.9%), orange crystals from petrol. (Found(HRESMS) m/z 419.954622. $C_{16}H_{17}Br_2NONa^+$ (^{79}Br) requires 419.955896). λ_{max} 202 (ε_{max} 8137), 195 (3850) nm. ^1H n.m.r. δ($CDCl_3$): 7.22-7.17, m, 5H, Ph; 6.81, s, ^{1}H.n.m.r:, C3-H; 2.38-2.36, m, 2H, C-4 chain,; 1.65-0.96, m, C4-chain. ^{13}C n.m.r. δ($CDCl_3$): 13.6, 22,3, 25.2, 29,5, 128.3, 128.8, 132.1, 139, 140.

3-Hexyl-5-dibromomethylene-1,5-dihydropyrrol-2-one

3-Hexyl-5-dibromomethylene-1,5-dihydropyrrol-2-one was prepared from 3-hexyl-5-dibromomethyl-5-hydroxy-1-phenyl-1,5-dihydropyrrol-2-one as

described above. Yellow granules from petrol. ν_{max}: 3378, 2957, 2925, 2854, 1692, 1598, 1501, 1492, 1445, 1122, 1081, 743, 677 cm-1. λ_{max}: 309 (ε_{max} 19,681) nm. ^{1}H n.m.r. δ ($CDCl_3$): 7.4-7.17, m, 5H, Ph; 2.37-2.34, m, 2H, C3-chain; 1.57-0.89, m, 13H, C5-chain.

1-Benzyl-3-butyl-5-dibromomethylene-1,5-dihydropyrrol-2-one

1-Benzyl-3-butyl-5-dibromomethyl-5-hydroxy-1,5-dihydropyrrol-2-one was dehydrated with P_2O_5 in $CHCl_3$ at room temperature for 72 hrs. The mixture was filtered through celite and the solvent evaporated in vacuo to yield a viscous oil, which solidified on keeping in the refrigerator. The solid was recrystallised from methanol/water to yield 1-benzyl-3-butyl-5-dibromomethylene-1,5-dihydropyrrol-2-one as colourless plates, m.p. 56-58°C (91%). ν_{max}: 2954, 1706, 1626, 1495, 1453, 1494, 1435, 1386, 1352, 1269, 1235, 1095, 765 cm-1. λ_{max}: 324 (ε_{max} 5985), 283 (16,201), 206 (10,972) nm.^{1}H n.m.r. δ($CDCl_3$): 7.3-7.07, m, 5H, Ph; 5.26, s, C3-H, 2.4-2.36, m, 2H, C4-chain; 1.6-0.95, m, C4-chain. ^{13}C n.m.r. δ($CDCl_3$): 13.7, 22, 25, 29.6, 44.2, 74.7, 89.25, 126, 127, 128, 132, 137.8, 138.8, 140, 172.1

1-Benzyl-5-dibromomethylene-3-hexyl-1,5-dihydropyrrol-2-one

This compound was prepared according to the procedure described for 1-benzyl-3-butyl-5-dibromomethylene-1,5-dihydropyrrol-2-one. ν_{max}: 2960, 2848, 2923, 2854, 1696, 1592, 1496, 1453, 1354, 1316, 977, 830, 738, 630 cm^{-1}. ^{1}H n.m.r. $\delta$$CDCl_3$): 7.3-7.08, m, 5H, Ph; 7.26, s, 2H, -C$\underline{H_2}$Ph; 5.26, s, C4-H; 2.4-2.36, m, 2H, C3-chain; 1.56-1.32, m, C3-chain.

1-Butyl-5-dibromomethylene-3-hexyl-1,5-dihydropyrrol-2-one

This compound was prepared according to the procedure described for 1-benzyl-3-butyl-5-dibromomethylene-1,5-dihydropyrrol-2-one. Yield (30%). ν_{max}: 2956, 2928, 2858, 1705, 1586, 1452, 1360, 1335, 1194, 1135, 1058, 846, 829 741 cm^{-1} ; λ_{max}: 290 (ε_{max} 18,927), 203 (9,409) nm. ^{1}H n.m.r. δ($CDCl_3$): 7.0, s, 1H, C4-H,; 3.99-3.93, t, 2H, -CH_2N-; 2.3, t, -CH_2- chain; 1.56-0.88, m, 20H, chain. ^{13}C n.m.r. δ($CDCl_3$): 13.7, 14, 19.7, 22.4, 25, 27, 29, 31.4, 32.1, 40.6, 132, 137, 139, 140.6, 172.

5-Dibromomethylene-3-hexyl-1,5-dihydropyrrol-2-one

This product was prepared by the dehydration of 5-dibromomethyl-3-hexyl-5-hydroxy-1,5-dihydropyrrol-2-one as described above, m.p. 103-105°.

5-Ethylidene-4-methyl-1,5-dihydropyrrol-2-one

5-Ethyl-5-hydroxy-4-methyl-2(5H)pyrrolinone was dehydrated to 5-ethylidene-4-methyl-1,5-dihydropyrrol-2-one with P_2O_5 in dichloromethane. ν_{max}: 3158, 3093, 3036, 1670, 1495, 1434, 1397, 1381, 1356, 1279, 956, 867, 796, 639. λ_{max}: 173 (ε_{max} 33,010) nm. ^{1}H n.m.r. δ($CDCl_3$): 8.94, s, 1H, -NH,; 5.85, 1H, s, C3-H; 5.33, q, J 7.53 Hz, -CH2=; 2.1, s, 3H, C3-Me; 1.92, d, J 7.53, C5-Me-CH=. ^{13}C n.m.r. δ($CDCl_3$): 11.7, 12.9, 107, 120.5, 140, 148, 172.

1-Benzyl-5-ethylidene-4-methyl-1,5-dihydropyrrol-2-one

1-Benzyl-5-ethylidene-4-methyl-1,5-dihydropyrrol-2-one was prepared by the dehydration of 1-benzyl-5-ethyl-5-hydroxy-4-methyl-1,5-dihydropyrrol-2-one as described before. λ_{max}: 206 (ε_{max} 2132) nm.

5-Bromomethylene-4-heptyl-1-phenyl-1,5-dihydropyrrol-2-one

p-Toluenesulfonic acid (0.05g) was added to a solution of 5-bromomethyl-5-hydroxy-4-heptyl-1-phenyl-1,5-dihydropyrrol-2-one in toluene. The mixture was refluxed for 1/2h and after cooling, washed with sat. $NaHCO_3$. The organic phase was dried over Na_2SO_4, and evaporated to yield an E,Z mixture of 5-bromomethylene-4-heptyl-1-phenyl-1,5-dihydropyrrol-2-one as a colourless oil which solidified on standing, m.p. 63-65°. ν_{max}: 3414, 3080, 2952, 2853, 1695, 1627, 1597, 1499, 1446, 1382, 1269, 1074, 907, 831 cm^{-1}; λ_{max}: 317 (ε_{max} 22,834), 278 (43,910), 204 (46,925) nm; ^{1}H n.m.r. δ($CDCl_3$): 7.4-7.24, m, 5H, Ph, 6.08-6.0 and 5.94-5.93, 2 d, two1H, C3-H; 2.85,m, 2H and 2.45, m, 2H, chain; 1.65-0.9, m, 13H, chain.

1-Benzyl-5-bromomethylene-4-heptyl-1,5-dihydropyrrol-2-one

1-Benzyl-5-bromomethyl-4-hepyl-5-hydroxy-1,5-dihydropyrrol-2-one dehydrated smoothly to an E and Z mixture of 1-benzyl-5-bromomethylene-4-heptyl-1,5-dihydropyrrol-2-one upon heating a solution of 1-benzyl-5-bromomethyl-4-heptyl-5-hydroxy-1,5-dihydropyrrol-2-one with p-toluenesulfonic acid in toluene; m.p. 52-55°; ν_{max}: 3096, 2927, 2857, 1704, 1630, 1387, 1357, 954, 855, 843 cm^{-1};λ_{max}: 319 (ε_{max} 10,220), 276 (19,433), 206 (17,040) nm; ^{1}H

n.m.r. δ($CDCl_3$): 7.29-7.15, m, 5H, Ph; 6.15 and 5.98, 2s, C4-H; 5.25 and 4.79, 2s, =CHBr; 2.72 and 2.39, m, chain; 1.7-0.89, m, 13H, chain.

Synthesis of 5-phenylaminomethylene-2(5H)furanone

4-Bromo-5-phenylaminomethylene-2(5H)furanone

A solution of 4-bromo-5-bromomethylene-2(5H)-furanone (0.30 g; 0.79 mmol) was dissolved in aniline (5 ml), and left to stand at room temperature for 3 hrs, during which time the mixture solidified. The solid was triturated with CH_2Cl_2/petrol (1:1; v/v, 20 ml) and filtered. The resulting solid was dried and recrystallised from ethanol to yield 4-bromo-5-phenylaminomethylene-2(5H)furanone (0.24g, 49%) as yellow needles, m.p. 200-202°C (decomp). (Found (HRESMS) m/z 287.963053. $C_{11}H_8BrNO_2Na^+$ (^{79}Br) requires m/z 287.963840). ν_{max} 3233, 3127, 1730, 1697, 1595, 1498, 1276, 1195, 932, 798, 756 cm^{-1}. λ_{max} 397 nm (ε_{max} 50,686); 246 (12,769), 202 (15,961). ^1H n.m.r. δ ($CDCl_3$): 9.99, d, J 10.44 Hz, 1H, -NHPh; 7.31-6.99, m, Ph; 7.07, d, J 10.44 Hz, 1H, =C<u>H</u>NHPh; 6.16, s, C3-H. ^{13}C n.m.r. δ ($CDCl_3$): 109.0, 116.2, 117.9, 129.9, 129.8, 133.9, 167.5.

5-Phenylaminomethylene-4-bromo-3-butyl-2(5H)-furanone

A solution of 4-bromo-3-butyl-5-bromomethylene-2(5H)-furanone (0.25 g; 0.81 mmol) in aniline (0.082 g; 0.88 mmol) was left to stand at room temperature for 72 h. The mixture was diluted with CH_2Cl_2 (50 ml), washed with aqueous hydrochloric acid (2M) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo leaving behind a brown viscous oil (0.29 g). The crude product was chromatographed on silica using dichloromethane to yield 5-phenylaminomethylene-4-bromo-3-butyl-2(5H)-furanone as a yellow solid. ^1H n.m.r δ($CDCl_3$): 7.40-6.80, m, 5H, Ph; 6.70, d J 12.5 Hz, =CH(N<u>H</u>)Ph; 2.42-2.40, m, 2H, CH_2-chain; 1.7-1.2, m, 4H, CH2-chain; 0.95, t J 7.3 Hz, CH3. (Found (HRESMS) m/z 344.021931. $C_{15}H_{16}BrNO_2Na^+$ (^{79}Br) requires m/z 344.021891).

4-Bromo-5-phenylaminomethylene-3-hexyl-2(5H)furanone

A mixture of 4-bromo-3-hexyl-5-bromomethylene-2(5H)-furanone (0.50g; 1.48 mmol) and aniline (1ml) in ethanol (10 ml) was heated at reflux for 2 h. After

cooling to room temperature, the mixture was evaporated to dryness and the residue extracted with dichloromethane (20 ml). The organic phase was washed with aqueous hydrochloric acid (2M) and dried over anhydrous sodium sulfate. Removal of the solvent and recrystallisation of the solid from light petroleum gave 3-bromo-5-phenylaminomethylene-3-hexyl-2(5H)furanone (0.50g; 100%) as yellow needles; m.p. 147-148°C. $\nu_{max:}$ 3242, 3161, 3109, 29212, 2842, 1728, 1683, 1600, 1581, 1500, 1350, 1236, 1055, 960, 750, 673 cm-1. λmax: 394 (ε_{max} 26,287) , 247 (8002) nm. ^{1}H n.m.r.δ($CDCl_3$): 7.32-6.97, m, 5H, Ph: 6.98, s, -N<u>H</u>Ph; 6.73, s, C5 =C<u>H</u>-NHPh; 2.4, t, -CH_2-chain; 1.61-0.88, m, 11H, chain. ^{13}C n.m.r.δ($CDCl_3$): 14.0, 22.0, 24.8, 27.6, 29, 31.0, 103.0, 113.0, 115.0, 122.5, 124.0, 129.5, 129.5, 131.0, 139.9, 167.0.

5-Phenylaminomethylene-4-heptyl-2(5H)furanone

5-Phenylaminomethyl-4-heptyl-5-hydroxy-2(5H)pyrrolinone

5-Bromomethylene-4-heptyl-2(5H)furanone (0.44g; 1.61 mmol) was dissolved in dry aniline (2 ml) and left to stand at room temperature for 24 h. Dichloromethane (10 ml) was added to the reaction mixture and the organic phase was washed with aqueous hydrochloric (2M) followed by water. After drying over sodium sulfate, the solvent was evaporated off to yield a pale yellow solid. The crude product was chromatographed on silica column using dichloromethane followed by CH_2Cl_2/EtOAc (2:1; v:v) as the eluents to yield 5-phenylaminomethyl-4-heptyl-6-hydroxy-2(5H)furanone (0.43g; 88.1%) as a pale yellow solid, m.p. 172-174°C. ν_{max}: 3192, 3037, 2957, 2931, 2953, 1676, 1643, 1598, 1502, 1493, 1336, 1250, 1160, 923, 757 cm^{-1}. λ_{max} 278 (ε_{max} 7188), 203 (8609) nm. ^{1}H n.m.r. δ($CDCl_3$): 7.53-7.25, 6H, Ph and -NHPh; 5.73, s, 1H,C3-H; 5.11,s, 1H, C5-OH; 3.37, d, 2H, -<u>CH_2</u>NHPh; 2.2-2.0, m, 2H, -CH_2-chain; 1.25-0.91, m, 13H, chain. ^{13}C n.m.r.δ($CDCl_3$): 14.0, 22.6, 25.5, 25.3, 25.8, 29.0, 29.2, 30.4, 31.6, 93.4, 121.8, 126.0, 126.7, 129.0, 134.6, 163.0, 170.4

5-Phenylaminomethylene-4-heptyl-2(5H)furanone

A sample of 5-phenylaminomethyl-4-heptyl-5-hydroxy-2(5H)furanone was dehydrated using p-toluenesulfonic acid in toluene to yield an E and Z mixture of 5-phenylaminomethylene-4-heptyl-2(5H)furanone as a colourless oil which solidified on standing in the fridge. ν_{max}: 3088(-NH), 3052, 2927, 2856, 1712, 1626, 1598, 1499, 1454, 1264, 1195, 759, 699 cm$^{-1\cdot}$ λ_{max} 292 (ε_{max} 7623), 204 (4728) nm. ^{1}H n.m.r. δ($CDCl_3$): 7.4-7.25, 6H, Ph and –N<u>H</u>Ph; 6.19-6.1, d, 1H,

C3-H; 5.93-6.0, 1H, d, C5- =C<u>H</u>NHPh; 1.68-0.90, 15H, chain. ^{13}C n.m.r.δ($CDCl_3$): 14, 22.5, 26.4, 28.1, 28.9, 29.0, 29.2, 30.0, 31.6, 31.7, 88.7, 93.0, 118.5, 122.6, 127.8, 128.2, 128.4, 128.6, 128.7, 129.3, 129.5, 134.0, 135.0, 142.0, 143.0, 152.0, 153.2, 168.0.

Synthesis of 5-arylamino and arylalkylamino-2(5H)furanones
4-bromo-5-benzylamino-5-bromomethyl-2(5H)furanone

Benzyl amine (0.10 g; 0.95 mmol) was added with stirring to an ice-cooled solution of the 4-bromo-5-(bromomethylene)-2(5H)furanone (0.16 g; 0.64 mmol) in dichloromethane (10 ml). The mixture was stirred at room temperature for 2.5 h, washed with aqueous hydrochloric acid solution (1M, 10 ml), dried (Na_2SO_4), and evaporated to yield a brown oil. The crude product was chromatographed on silica using dichloromethane/ethyl acetate (1:4; v:v) as the eluent and recrystallised from dichloromethane/light petroleum to yield *4-bromo-5-benzylamino-5-bromomethyl-2(5H)furanone* as orange flakes. m.p. 137-139 ° (Found (HRESMS) m/z 381.901032. $C_{12}H11Br_2NO_2Na^+$ (^{79}Br) requires 381.904812). ν_{max} 3256, 1674, 1655, 1431, 1413, 1352, 1072, 1054, 699 cm^{-1}. λ_{max} 257 (ε_{max} 2879) nm. ^1H n.m.r. δ ($CDCl_3$): 7.38,d, J 11 Hz, 1H,-$NHCH_2$-; 7.37-7.29, m, Ph; 6.38, s, C3-H, 4.65, d, J 15 Hz, 1H, -CH_2Br; 4.44, d, J 15 Hz, 1H, -CH_2Br and 3.58-3.44, dd, J 15 Hz. ^{13}C n.m.r.δ ($CDCl_3$): 30.6, 42.8, 53.0, 92.2, 128.0, 128.2, 128.9, 137.0, 142.0, 168.0.

4-Bromo-5-benzylamino-5-bromomethyl-3-hexyl-2(5H)furanone

Benzylamine (0.32g; 2.96 mmol) was added with stirring to a solution of 4-bromo-3-hexyl-5-bromomethylene-2(5H)-furanone (0.50 g; 1.48 mmol) in ethanol (6 ml). The mixture was stirred at room temperature for 1 h and evaporated to dryness. The residue was extracted with dichloromethane (20 ml) and the dichloromethane extract washed with aqueous hydrochloric acid (2M). After drying over anhydrous sodium sulfate, removal of the solvent gave a thick viscous oil. Column chromatography on silica gel using dichloromethane followed by dichloromethane/ethyl acetate (19:1) as the eluents afforded 4-bromo-5-benzylamino-5-bromomethyl-3-hexyl-2(5H)furanone (0.36g; 56%) as a viscous oil; m.p. 72-75°.ν_{max}3277, 3065, 3032, 2954, 2928, 2857,1681, 1496, 1411, 1355, 1151, 1064, 1104, 1030, 988, 907, 726, 698. λ_{max}: 277 (ε_{max}: 39,542), 205 (38,034) nm. ^1H n.m.r. δ($CDCl_3$): 7.4-7.26, m, Ph; 4.8-4.74, d, and 4.4, d, C5-CH_2Br; 3.6 and 3.53, d, C5-NHC<u>H</u>$_2$Ph; 2.42-2.33. m. –CH_2, chain;

1.56-0.85, m, 11H, chain. ^{13}C n.m.r. δ($CDCl_3$): 22.0, 25.0, 27.0, 28.8, 31.4, 42.9, 46.7, 49.5, 90.6, 91.6, 127.0, 128.0, 129.0, 136.0, 136.7, 136.9, 138.0, 140.0, 144.0, 168.0, 170.6.

5-Phenylamino-3,5-dimethyl-2(5H)-furanone

Method A

A solution of 3,5-dimethyl-5-hydroxy-2(5H)-furanone (0.13g; 1.02 mmol) in dry aniline (2 mls) was stirred at room temperature for 1 hr. A thin layer chromatography analysis of the mixture (developing solvent; CH_2Cl_2) indicated completion of the reaction as indicated by the disappearance of the starting material. Dichloromethane (25 mls) were added to the mixture and the solution washed with aqueous hydrochloric acid solution (1M; 3 x 20 mls). The organic layer was dried over anhydrous sodium sulfate and evaporated to yield 5-phenylamino-3,5-dimethyl-2(5H)-furanone as a viscous oil which solidified on keeping (0.013 g). A sample was recrystallised from dichloromethane/light petroleum to yield the furanone as colourless needles m.p. 86-88°C ° ν_{max} 3324, 3063, 2965, 1757, 1670, 1588, 1532, 1434, 1122, 1081, 1009, 892, 779, 682 cm^{-1}. λ_{max} 254 nm (ε_{max} 34,118). ^1H n.m.r. δ ($CDCl_3$): 7.52-7.08, m, 2H Ph; 7.26, d, 1H, J 6.15 Hz, -$CH_2\underline{NH}Ph$; 4.78, q, J 6.15 Hz, -$C\underline{H_2}NHPh$, 1.30, d, -Me. ^{13}Cn.m.r. δ ($CDCl_3$): 118.6, 27.0, 55.5, 108.1, 116.5, 121.0, 122.4, 125.2, 129.0, 129.3, 129.4, 132.6, 136.6, 141.5, 166.0.

Method B

A mixture of 3,5-dimethyl-5-hydroxy-2(5H)-furanone (0.13g; 1.02 mmol) and aniline (2 ml) in dry toluene (10 ml) was refluxed for 5h. The mixture was cooled and evaporated. The residue was dissolved in dichloromethane (25 ml) and the solution washed with aqueous hydrochloric acid solution (1M; 3 x 20 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to yield 5-phenylamino-3,5-dimethyl-2(5H)-furanone as a viscous oil. The crude product was chromatographed on silica using dichloromethane/ethyl acetate (19:1) as the eluent (Yield 58.0%).

5-Phenylamino-5-methyl-4-phenyl-2(5H)-furanone

A mixture of 5-hydroxy-5-methyl-4-phenyl-2(5H)-furanone (0.13g; 1.02 mmol) and aniline (2 ml) in dry toluene (10 ml) was refluxed for 5h. The mixture was cooled and washed with aqueous hydrochloric acid solution (2M; 3 x 20 mls).

The organic layer was dried over anhydrous sodium sulfate and evaporated to yield a viscous oil. The crude product was chromatographed on silica using dichloromethane/ethyl acetate (19:1) as the eluent and recrystallised from dichloromethane/light petroleum to yield 5-phenylamino-5-methyl-4-phenyl-2(5H)-furanone (0.10 g; 72%) as colourless flakes. m.p. 158-160°Cνmax: 3355, 1724, 1608, 1534, 1501, 1320, 1291, 1376, 1030, 943, 846, 770, 756, 691, 639. λ_{max} 276(ε_{max} 7056), 238 (5615)nm. ^{1}H n.m.r. δ($CDCl_3$): 7.94-7.44, m, 5H, -NH<u>Ph</u>; 7.14-6.82, m, 5H, C4-Ph; 6.4, s, 1H, C3-H; 4.53, s, 1H, -N<u>H</u>Ph; 1.9, s, C5-Me. ^{13}C n.m.r.δ($CDCl_3$): 117.3, 120, 122,5, 128, 129,5, 131, 142, 159, 166, 170.

5-Benzylamino-5-benzylaminomethyl-3-methyl-2(5H)pyrrolinone

A mixture of 3,5-dimethyl-5-hydroxy-2(5H)-furanone (0.50g; 2.63 mmol) and benzyl amine (2g, 18.7 mmol) in dry toluene (10 ml) was refluxed for 5h. The mixture was cooled in an ice-bath, and the resulting solid was filtered and washed with dichloromethane/light petroleum (1:1). The product was dried to yield *5-benzylamino-5-benzylaminomethyl-3-methyl-2(5H)pyrrolinone* as colourless needles (Xg, 30%), m.p. 210-212°C. ν_{max} 3287, 3052, 2935, 2873, 1631, 1542, 1455, 1363, 1209, 1024, 758, 694 cm^{-1}. ^{1}H N.M.R. δ ($CDCl_3$): 8.28, t, 1H, -N<u>H</u>Ph; 7.22, m, 11 H, Ph and –N<u>H</u>Ph; 4.23, m, 2 –CH_2Ph; 3.27, s, C5-C<u>H_2</u>Ph; 2.8-2.13, m, C5-C<u>H_2</u>NHPh; 1.04, d, 3H, C3-Me. ^{13}C n.m.r. (d_6-DMSO): 18.2, 40.5, 39.1, 39.4, 42.3, 42.4, 126.9, 127.0, 127.3, 127.5, 128.6, 140, 140.1, 171.0, 175.3.

5-Benzylaminomethyl-3-methyl-2(5H)furanone

Phosphorus pentoxide (2g) was added to a solution of 3,5-dimethyl-5-hydroxy-2(5H)-furanone (0.50g; 2.15 mmol) in dichloromethane (25 ml). The mixture was refluxed for 2h and the cooled solution was filtered through celite and evaporated in vacuo to yield 3-methyl-5-methylene-2(5H)-furanone as a colourless oil (0.37 g; 82%). The methylene product was dissolved in dichloromethane (5 ml) and benzylamine (1.15 g; 10.8 mmol) was added at room temperature. The mixture was stirred at room temperature for 1 h. After evaporation of the solvent the crude product was chromatographed on silica using dichloromethane/light petroleum as the eluent to yield *5-benzylaminomethyl-3-methyl2(5H)furanone* as a colourless oil (0.12 g; 26%). ν_{max}: 2929, 2854, 1788, 1747, 1715, 1618, 1456, 1388, 1373, 845, 712 cm^{-1}.

λ_{max}: 308 nm (ε_{max} 1462), 260 (5243).[1]H n.m.r.δ ($CDCl_3$): 7.29-7.21, m, 6H, Ph and –N<u>H</u>CH_2Ph; 6.65, s, 1H, C_4-H; 4.82,s, 2H, -<u>CH_2</u>Ph; 4.70, d, -C<u>H_2</u>NHPh; 2.02, s, C_3-Me. ^{13}C n.m.r. δ ($CDCl_3$): 10.8, 25.9, 42.9, 95.0, 105.3, 126.9, 127.1, 128.5, 131.2, 134.2, 137.2, 148.4.

Side-chain functionalization

3-(1'-Bromohexyl)-1-Butyl-5-dibromomethylene-1,5-dihydropyrrol-2-one

N-Bromosuccinimide (0.32g; 1.79 mmol) was added to a solution of 1-butyl-5-dibromomethyl-3-hexyl-1,5-dihydropyrrol-2-one (0.64 g; 1.63 mmol) containing few crystals of benzoyl peroxide in CCl_4 (25 ml). The mixture was heated at reflux under a 100 watt fluorescent lamp for 24 h. The reaction mixture was cooled and passed through a pad of Celite. The filtrate was evaporated to dryness to yield a brown oil which was chromatographed on a silica column using CH_2Cl_2/petrol (1:1) as the eluent to yield 3-(1'-Bromohexyl)-1-Butyl-5-dibromomethylene-1,5-dihydropyrrol-2-one (0.46 g; 59.8%) as a pale yellow oil. (Found): HRESMS): m/z 483.849575. $C_{15}H_{14}NB_4O_3$) requires 483.851758. ν_{max}: 3017,2950, 1709, 1598, 1593, 1480, 1215, 1194, 845, 695, 668 cm^{-1}. λ_{max}: 326 (ε_{max} 4070) nm. ^{1}H n.m.r. δ($CDCl_3$): 7.48-7.4, m, 5H,Ph; 4.88-4.84, t, -C<u>H</u>Br-chain; 2.19-2.11,m, -CH_2-chain; 1.53-0.98, m, 6H, chain. ^{13}C n.m.r. δ($CDCl_3$): 13.12, 20.95, 26.8, 39, 43.9, 79.5, 95, 128.6, 128.9, 129.4, 133.8, 134.5, 138.2, 139.6, 168.7

3-(1'-Bromobutyl)-1-butyl-5-dibromomethylene-1,5-dihydropyrrol-2-one

N-Bromosuccinimide (0.32g; 1.79 mmol) was added to a solution of N-butyl-5-dibromomethyl-3-hexyl-2(5H)pyrrolinone (0.64 g; 1.63 mmol) containing few crystals of benzoyl peroxide (0.01g) in CCl_4 (25 ml). The mixture was heated at reflux under a 100 watt fluorescent lamp for 24 h. The reaction mixture was cooled and passed through a pad of Celite. The filtrate was evaporated to dryness to yield a semi-solid (0.69g) which was chromatographed on a silica column using CH_2Cl_2/petrol (1:1) as the eluent to yield 3-(1'-bromobutyl)-1-butyl-5-dibromomethylene-1,5-dihydropyrrol-2-one (0.46g; 60%) as a pale yellow oil. λ_{max}: 2930, 2957, 2871, 1705, 1584, 1357, 1192, 1055, 902, 769, 651 cm^{-1}; λ_{max}: 325 (ε_{max} 11,669), 202 (9,879) nm. 1H n,m,r, δ($CDCl_3$): 7.29, d, 1H, C4-H; 4.78, t, 1H, C3-C<u>H</u>Br- chain; 3.98,t, 2H, >NC<u>H_2</u>-; 2.10, m, -CH_2-chain; 1.58-0.93, m, 16H, chain; 13C n.m.r. δ($CDCl_3$): 13.78, 13.96, 19,8, 22.4, 27.4, 31, 32.2, 37, 41, 43.9, 98.7, 132.5, 138.2, 140, 169.

3-(1'-Bromobutyl)-5-dibromomethylene- N-phenyl-1,5-dihydropyrrol-2-one
N-Bromosuccinimide (0.056g; 0.316 mmol) was added to a solution of 5-dibromomethyl-3-butyl-1-phenyl-1,5-dihydropyrrol-2-one (0.64 g; 1.63 mmol) containing few crystals of benzoyl peroxide (0.01g) in CCl_4 (10 ml). The mixture was heated at reflux under a 100 watt fluorescent lamp for 24 h. The reaction mixture was cooled and passed through a pad of Celite. The filtrate was evaporated to dryness to yield a brown oil (0.17g) which was chromatographed on a silica column using CH_2Cl_2/petrol (1:1) as the eluent to yield 3-(1'-bromobutyl)-5-dibromomethylene-1-phenyl-1,5-dihydropyrrol-2-one as a pale viscous oil (0.10g). (Found:HRESMS) m/z: 483.849575, $C_{15}H_{14}Br_3NONa^+$ (Br^{79}) requires 483.851758. ν_{max}: 3017, 2950, 1709, 1598, 1593, 1480, 1215, 1194, 1122, 845, 756, 695, 668 cm^{-1}.λ_{max}: 326 (ε_{max} 3,896), 202 (5,566) nm. 1H n.m.r. $\delta CDCl_3$): 7.45, m, 6H, Ph and C3-H; 4.86, t, 1H, C3-C<u>H</u>Br- chain; 2.16, m, $-CH_2$ chain; 1.53-0.98, m, 7H, chain. ^{13}C n.m.r.$\delta(CDCl_3)$: 13, 21, 26.8, 39, 43, 79.5, 95, 107, 128.6, 129.4, 134, 134.5, 138, 139.6, 169.

<u>**Biological activity of furanones**</u>

Effect of furanones as inhibitor of AHL-mediated quorum sensing, AI-2 pathway and growth of *S. aureus*
Methods
Gfp assay
Briefly, the Gfp assay determines the relative effectiveness of a compound as an inhibitor of AHL mediated quorum sensing. The assay is dependent on a bacterial strain that carries a reporter plasmid. This plasmid expresses the green fluorescent protein (Gfp) in the presence of AHLs (2). The presence of a competitor will prevent AHL mediated Gfp expression of the reporter. The assay can be used to generate an index of inhibition for each compound. The results here, presented as good, moderate, or poor, are based on the index of each of the compounds as an inhibitor of AHL mediated quorum sensing using this bioassay.

Attachment/Biofilm formation
The ability of furanones to inhibit biofilm formation or attachment has been determined using a modification of the 96 well microtitre method described by

Christensen et al. ((1)). The furanones are added to the wells of the microplate and the solvent is allowed to evaporate, leaving the furanones adsorbed onto the plate. Then a suspension of the monitor bacterium, *Pseudomonas aeruginosa*, is added to each well and incubated for 24h. Following incubation, the wells are rinsed to remove unattached or loosely adhered cells. The attached wells are fixed with formaldehyde and subsequently stained with crystal violet. Following extensive washing to remove the crystal violet, the wells are read at 600 nm. The attachment/biofilm formation in the presence of the furanones is calculated as the percentage of the controls, which are not exposed to the furanones.

Two-Component signal transduction Assays

Taz-1 Assay

The Taz-assay carried out according to the method of Jin and Inouye (1993) with the following alterations. *E. coli* RU1012 (pYT0301) were grown overnight in M9 medium at 37°C supplemented with 100 µg/ml ampicillin and 50 ug/ml kanamycin. This overnight culture was then used to inoculate 50 ml M9 medium in side-arm flasks which were then incubated at 37°C and shaken at 180 rpm. The OD_{610} of the growing cultures was monitored regularly and when the $OD_{610} = 0.2$ the cultures were placed on ice. Aspartate was added to side-arm flasks to give a final concentration of 3 mM (aspartate stock solution made up in M9 salts).

The test compound or mixtures of compounds were dissolved in ethanol and added to cultures to give the required final concentrations. Negative controls were prepared with equal volumes of ethanol. Cultures were then placed in a 37°C incubator and shaken for 4 hours (OD_{610} approximately 0.7) before being removed and put on ice. Samples were then removed for □eta-galactosidase assays carried out according to the method of Miller (1972).

***V. harveyi* bioassay for the detection of AI-2 activity**

The *V. harveyi* bioassay was performed as described previously (Surette and Bassler, 1998). The *V. harveyi* reporter strain BB170 was grown for 16 hours at 30°C with shaking in AB medium. Cells were diluted 1:5,000 into 30°C prewarmed AB medium and 90 µl of the diluted suspension was added to wells containing supernatant. Furanones were added to the wells to achieve the

desired final concentrations and the final volume in each well was adjusted with sterile medium to 100 µl. Ten µl of *V. harveyi* BB152 (AI-1-, AI-2+) supernatant was used as a positive control and 10 µl of *E. coli* DH5α supernatant or sterile media was used as a negative control. This strain of *E. coli* has previously been shown to harbor a mutation in the AI-2 synthase gene, *ygaG,* which results in a truncated protein with no AI-2 activity (Surette et al. 1998). The microtiter plates were incubated at 30°C with shaking at 175 rpm. Hourly determinations of the total luminescence were quantified using the chemiluminescent setting on a Wallac (Gaithersburg, MD) model 1450 Microbeta Plus liquid scintillation counter. The *V. harveyi* cell density was monitored by the use of a microplate reader (Bio-Rad, Hercules, CA). Activity is reported as the percentage of activity obtained from *V. harveyi* BB152 cell-free supernatant. While the absolute values of luminescence varied considerably between experiments, the pattern of results obtained was reproducible.

Growth of *Staphylococcus aureus*

Material and methods

The growth of *Staphylococcus aureus* against furanones was tested in sidearm flasks. One percent of an overnight culture was added to the growth media, Nutrient Broth, containing furanones at the concentrations 1-50 µg/ml. The bacteria were incubated at 37C and growth was measured at 610 nm.

The results of these experiments are summarised in the Table 1.

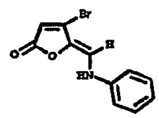
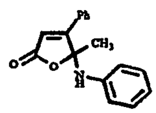
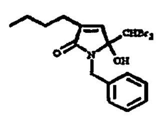
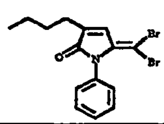
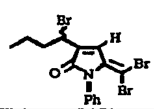
Compound	AHL	AI-2 (% of control)	S. aureus (% of control)
	++	2 %, 50 μg/ml 93%, 10 μg/ml	NE at 50 μg/ml
	+++	26%, 50μg/ml 57%, 10ug/ml 80%, 5ug/ml	NE at 50μg/ml
	+++	21 %, 50μg/ml	NE at 50μg/ml
	+++		NE at 50μg/ml
	++++		0% growth at 25μg/ml for 10hrs

Table 1. Summary of activity for lactam and other N containing analogues as inhibitor of AHL-mediated quorum sensing, AI-2 pathway and growth of S. aureus.

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Any description of prior art documents herein is not to be taken as an admission that the documents form part of the common general knowledge of the relevant art.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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